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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03101828.6

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Freeze-dried pharmaceutical formulations containing FSH and LH

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Bemerkungen:
Remarks: See page 1 of the description for the original title.
Remarques:

FSH and LH pharmaceutical formulations

Field of Invention

The invention relates to the field of freeze dried formulations of follicle-stimulating hormone (FSH), luteinising hormone (LH), and mixtures of FSH and luteinising hormone (LH), and to methods of producing such formulations.

Background of the invention

Follicle-stimulating hormone (FSH), luteinising hormone (LH) and chorionic gonadotrophin (CG) are injectable proteins falling into the class of gonadotrophins. FSH, LH and hCG are used alone and in combination in the treatment of infertility and reproductive disorders in both female and male patients.

In nature, FSH and LH are produced by the pituitary gland. For pharmaceutical use, FSH and LH and their variants may be produced recombinantly (rFSH and rLH), or they may be produced from the urine of postmenopausal women (uFSH and uLH).

FSH is used in female patients in ovulation induction (OI) and in controlled ovarian hyperstimulation (COH) for assisted reproductive technologies (ART). In a typical treatment regimen for ovulation induction, a patient is administered daily injections of FSH or a variant (about 75 to 300 IU FSH/day) for a period of from about 6 to about 12 days. In a typical treatment regimen for controlled ovarian hyperstimulation, a patient is administered daily injections of FSH or a variant (about 150-600 IU FSH/day) for a period of from about 6 to about 12 days.

FSH is also used to induce spermatogenesis in men suffering from oligospermia. A regimen using 150 IU FSH 3 times weekly in combination with 2'500 IU hCG twice weekly has been successful in achieving an improvement in sperm count in men suffering from hypogonadotrophic hypogonadism [1].

LH is used in female patients in combination with FSH in OI and in COH, particularly in those patients having very low endogenous LH levels or resistance to LH, such as women suffering from hypogonadotrophic hypogonadism (HH, WHO group I) or older patients (i.e. 35 years or older), and patients in which embryo implantation or early miscarriage is a problem. LH in combination with FSH has traditionally been available in a preparation called human menopausal gonadotrophins (hMG)

extracted from the urine of postmenopausal women. hMG has a 1:1 ratio of FSH:LH activity.

CG acts at the same receptor as LH and elicits the same responses. CG has a longer circulation half-life than LH and is therefore commonly used as a long-acting source of LH-activity. CG is used in OI and COH regimens to mimic the natural LH peak and trigger ovulation. An injection of human chorionic gonadotrophin (hCG) is used to trigger ovulation at the end of stimulation with FSH or a mixture of FSH and LH. CG may also be used together with FSH during stimulation for OI and COH, in order to provide LH-activity during stimulation in patients in which LH-activity is desirable, such as those mentioned above.

FSH, LH and CG are members of the heterodimer, glycoprotein hormone family that also includes thyroid stimulating hormone (TSH). The members of this family are heterodimers, comprising an α- and a β-subunit. The subunits are held together by noncovalent interactions. The human FSH (hFSH) heterodimer consists of (i) a mature 92 amino acid glycoprotein alpha subunit, which also is common to the other human family members (i.e., chorionic gonadotrophin ("CG"), luteinising hormone ("LH") and thyroid stimulating hormone ("TSH"); and (ii) a mature 111 amino acid beta subunit that is unique to FSH[2]. The human LH heterodimer consists of (i) the mature 92 amino acid glycoprotein alpha subunit; and (ii) a mature 112 beta subunit that is unique to LH[3]. The alpha and beta subunits of the glycoproteins may be prone to dissociate in formulations, due to interaction with a preservative, surfactant and other excipients. Dissociation of the subunits leads to loss of biological potency[4].

FSH is formulated for intramuscular (IM) or subcutaneous (SC) injection. FSH is supplied in lyophilised (solid) form in vials or ampoules of 75 IU/vial and 150 IU/vial with a shelf life of one and a half to two years when stored at 2-25°C. A solution for injection is formed by reconstituting the lyophilised product with water for injection (WFI). For ovulation induction or controlled ovarian hyperstimulation, daily injections with starting doses of 75 IU to 600 IU are recommended for up to about ten days. Depending on the patient's response, up to three cycles of treatment with increasing doses of FSH can be used. With lyophilised formulations, the patient is required to reconstitute a new vial of lyophilised material with diluent and administer it immediately after reconstitution on a daily basis [Package insert N1700101A, published in February 1996, for Fertinex™ (urofollitropin for injection, purified) for subcutaneous injection, by Serono Laboratories, Inc., Randolph, MA].

FSH has also been formulated in both single-dose and multi-dose liquid formats, in vials, or ampoules. Single dose formats must remain stable and potent in storage prior to use. Multi-dose formats must not only remain stable and potent in storage prior to use, but must also remain stable, potent and relatively free of bacteria over the multiple-dose use regimen administration period, after the seal of the ampoule has been compromised. For this reason, multi-dose formats contain a bacteriostatic agent.

LH is formulated for intramuscular (IM) or subcutaneous (SC) injection. LH is supplied in lyophilised (solid) form in vials or ampoules of 75 IU/vial with a shelf life of one and a half to two years when stored at 2-25°C. A solution for injection is formed by reconstituting the lyophilised product with water for injection (WFI). For ovulation induction or controlled ovarian hyperstimulation, in conjunction with FSH, daily injections with starting doses of 75 IU to 600 IU LH are recommended for up to about ten days.

EP 0 618 808 (Applied Research Systems ARS Holding N.V.) discloses a pharmaceutical composition comprising a solid intimate mixture of gonadotrophin and a stabilising amount of sucrose alone or in combination with glycine.

EP 0 814 841 (Applied Research Systems ARS Holding N.V.) discloses a stable, liquid pharmaceutical composition comprising recombinant human chorionic gonadotrophin (hCG) and a stabilizing amount of mannitol.

EP 0 448 146 (AKZO N.V.) discloses a stabilized gonadotrophin containing lyophilisate comprising one part by weight of a gonadotrophin; and 200 to 10,000 parts by weight of a dicarboxylic acid salt stabilizer associated with the gonadotrophin.

EP 0 853 945 (Akzo Nobel N.V.) discloses a liquid gonadotrophin-containing formulation characterised in that the formulation comprises a gonadotrophin and stabilising amounts of a polycarboxylic acid or a salt thereof and of a thioether compound.

WO 00/04913 (Eli Lilly and Co.) discloses a formulation comprising FSH or an FSH variant, containing an alpha and beta subunit, and a preservative selected from

the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent.

Summary of the invention

It is an object of the invention to provide new freeze dried formulations of FSH or FSH variants, or LH or LH variants, to provide methods for their preparation, and methods for their pharmaceutical or veterinary use in the treatment of fertility disorders.

It is a further object of the invention to provide new freeze dried formulations of mixtures of FSH and LH, to provide methods for their preparation, and methods for their pharmaceutical or veterinary use in the treatment of fertility disorders.

In a first aspect, the invention provides a freeze dried formulation comprising FSH or a variant thereof, and a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

In a second aspect, the invention provides a freeze dried formulation comprising LH or a variant thereof, and a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

In a third aspect, the invention provides a freeze dried formulation comprising FSH or a variant thereof as well as LH or a variant thereof, and a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

In a fourth aspect, the invention provides a method for manufacturing a freeze dried formulation comprising forming a mixture of FSH or a variant thereof with a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and subjecting the mixture to lyophilisation.

In a fifth aspect, the invention provides a method for manufacturing a freeze dried formulation comprising forming a mixture of LH or a variant thereof with a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and subjecting the mixture to lyophilisation.

In a sixth aspect, the invention provides a method for manufacturing a freeze dried formulation comprising forming a mixture of FSH or a variant thereof and LH or a variant thereof with a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and subjecting the mixture to lyophilisation.

In a seventh aspect, the invention provides an article of manufacture for human pharmaceutical use, comprising a first container comprising freeze dried FSH or an FSH variant, and a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and a second container comprising a solvent for reconstitution, preferably an aqueous solution containing a bacteriostatic, preferably m-cresol.

In an eight aspect, the invention provides an article of manufacture for human pharmaceutical use, comprising a first container comprising freeze dried LH or an LH variant, and a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and a second container comprising a solvent for reconstitution, preferably an aqueous solution containing a bacteriostatic, preferably m-cresol.

In an ninth aspect, the invention provides an article of manufacture for human pharmaceutical use, comprising a first container comprising freeze dried FSH as well as LH or an FSH or LH variant variant, and a surfactant selected from block copolymers of ethylene oxide and propylene oxide, preferably Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 and a second container comprising a solvent for reconstitution, preferably an aqueous solution with m-cresol.

Detailed description of the invention

The FSH, LH or FSH and LH formulations of the invention have improved or more suitable properties or stability, and are useful for infertility treatment in women and/or men. These formulations and articles of manufacture are additionally suitable for use in injectable and alternative delivery systems, e.g., but not limited to, nasal, pulmonary, transmucosal, transdermal, oral, subcutaneous, intramuscular or parenteral sustained release. In a particularly preferred embodiment the formulations of the invention are for subcutaneous and/or intramuscular injection. The FSH, LH or FSH and LH variant formulations provided may also have increased *in vivo* potency over time compared to known commercial products, by preventing or reducing loss of activity or stability, or by improving any aspect of the effectiveness or desirability of administration, e.g., by at least one of mode, frequency, dosage, comfort, ease of use, biological activity *in vitro* or *in vivo,* and the like.

Follicle stimulating hormone, or FSH, as used herein refers to the FSH produced as a full-length mature protein which includes, but is not limited to human FSH or "hFSH", whether produced recombinantly or isolated from human sources, such as the urine of postmenopausal women. The protein sequence of the human glycoprotein alpha subunit is provided in SEQ ID NO: 1, and the protein sequence of the human FSH beta subunit is given in SEQ ID NO:2.

The expression "FSH variant" is meant to encompass those molecules differing in amino acid sequence, glycosylation pattern or in inter-subunit linkage from human FSH but exhibiting FSH-activity. Examples include CTP-FSH, a long-acting modified recombinant FSH, consisting of the wild type α-subunit and a hybrid β-subunit in which the carboxy terminal peptide of hCG has been fused to the C-terminal of the β-subunit of FSH, as described in LaPolt *et al.*; Endocrinology; **1992**, *131*, 2514-2520; or Klein et al.; Development and characterization of a long-acting recombinant hFSH agonist; Human Reprod. 2003, 18, 50-56]. Also included is single chain CTP-FSH, a single chain molecule, consisting of the following sequences (from N-terminal to C-terminal):

βFSH	βhCG-CTP(113-145)	αFSH

wherein βFSH signifies the β-subunit of FSH, βhCG CTP (113-145) signifies the carboxy terminal peptide of hCG and αFSH signifies the α-subunit of FSH, as described by Klein *et al.*[5] Other examples of FSH variants include FSH molecules having additional glycosylation sites incorporated in the α- and/or β-subunit, as disclosed in WO 01/58493 (Maxygen), particularly as disclosed in claims 10 and 11 of WO 01/58493, and FSH molecules with intersubunit S-S bonds, as disclosed in WO 98/58957.

The FSH variants referred to herein also include the carboxy terminal deletions of the beta subunit that are shorter than the full length mature protein of SEQ ID NO:2.. Carboxy terminal deletions of the human beta subunit are provided in SEQ IDS NOS: 3, 4, and 5. It is understood that the carboxy terminal variants of the beta chain form dimers with a known alpha subunit to form an FSH variant heterodimer.

FSH heterodimers or FSH variant heterodimers can be produced by any suitable method, such as recombinantly, by isolation or purification from natural sources as may be the case, or by chemical synthesis, or any combination thereof.

The use of the term "recombinant" refers to preparations of FSH, LH or FSH and LH variants that are produced through the use of recombinant DNA technology (see for example WO 85/01958). The sequences for genomic and cDNA clones of FSH are known for the alpha and beta subunits of several species[6]. One example of a method of expressing FSH or LH using recombinant technology is by transfection of eukaryotic cells with the DNA sequences encoding an alpha and beta subunit of FSH or LH, whether provided on one vector or on two vectors with each subunit having a separate promoter, as described in European patent nos. EP 0 211 894 and EP 0 487 512. Another example of the use of recombinant technology to produce FSH or LH is by the use of homologous recombination to insert a heterologous regulatory segment in operative connection to endogenous sequences encoding the subunits of FSH or LH, as described in European patent no. EP 0 505 500 (Applied Research Systems ARS Holding NV).

The FSH or FSH variant used in accordance with the present invention may be produced not only by recombinant means, including from mammalian cells, but also may be purified from other biological sources, such as from urinary sources. Acceptable methodologies include those described in Hakola, K. Molecular and

Cellular Endocrinology, 127:59-69, 1997; Keene, et al., J. Biol. Chem., 264:4769-4775, 1989; Cerpa-Poljak, et al., Endocrinology, 132:351-356, 1993; Dias, et al., J. Biol. Chem., 269:25289-25294, 1994; Flack, et al., J. Biol. Chem., 269:14015-14020, 1994; and Valove, et al., Endocrinology, 135:2657-2661, 1994, U.S. Patent 3,119,740 and US Patent no. 5,767,067.

Luteinising hormone, or LH, as used herein refers to the LH produced as a full-length mature protein, which includes, but is not limited to human LH or "hLH", whether produced recombinantly or isolated from human sources, such as the urine of postmenopausal women. The protein sequence of the human glycoprotein alpha subunit is provided in SEQ ID NO: 1, and the protein sequence of the human LH beta subunit[7] is given in SEQ ID NO: 6. In a preferred embodiment the LH is recombinant.

The expression "LH variant" is meant to encompass those molecules differing in amino acid sequence, glycosylation pattern or in inter-subunit linkage from human LH but exhibiting LH-activity.

LH heterodimers or LH variant heterodimers can be produced by any suitable method, such as recombinantly, by isolation or purification from natural sources as may be the case, or by chemical synthesis, or any combination thereof.

The term "administer" or "administering" means to introduce a formula tion of the present invention into the body of a patient in need thereof to treat a disease or condition.

The term "patient" means a mammal that is treated for a disease or condition. Patients are of, but not limited to, the following origin, human, ovine, porcine, equine, bovine, rabbit and the like.

The term "potency" in relation to FSH activity, refers to the ability of an FSH formulation or a mixed formulation, to elicit biological responses associated with FSH, such as ovarian weight gain in the Steelman-Pohley assay[8], or follicular growth in a female patient. Follicular growth in a female patient can be evaluated by ultrasound, for example, in terms of the number of follicles having a mean diameter of at or about 16 mm on day 8 of stimulation. Biological activity is evaluated with respect to an accepted standard for FSH.

The term "potency" in relation to LH activity, refers to the ability of an LH formulation or a mixed formulation, to elicit biological responses associated with LH, such as seminal vesicle weight gain method.[9] Biological activity of LH is evaluated with respect to an accepted standard for LH.

The term "aqueous diluent" refers to a liquid solvent that contains water. Aqueous solvent systems may be consist solely of water, or may consist of water plus one or more miscible solvents, and may contain dissolved solutes such as sugars, buffers, salts or other excipients. The more commonly used non-aqueous solvents are the short-chain organic alcohols, such as, methanol, ethanol, propanol, short-chain ketones, such as acetone, and poly alcohols, such as glycerol.

An "isotonicity agent" is a compound that is physiologically tolerated and imparts a suitable tonicity to a formulation to prevent the net flow of water across cell membranes that are in contact with the formulation. Compounds such as glycerin, are commonly used for such purposes at known concentrations. Other suitable isotonicity agents include, but are not limited to, amino acids or proteins (e.g., glycine or albumin), salts (e.g., sodium chloride), and sugars (e.g., dextrose, sucrose and lactose).

The term "bacteriostatic" or "bacteriostatic agent" refers to a compound or compositions added to a formulation to act as an anti-bacterial agent. A preserved FSH or FSH variant or FSH and LH containing formulation of the present invention preferably meets statutory or regulatory guidelines for preservative effectiveness to be a commercially viable multi-use product, preferably in humans. Examples of bacteriostatics include phenol, *m*-cresol, *p*-cresol, *o*-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal.

The term "buffer" or "physiologically-acceptable buffer" refers to solutions of compounds that are known to be safe for pharmaceutical or veterinary use in formulations and that have the effect of maintaining or controlling the pH of the formulation in the pH range desired for the formulation. Acceptable buffers for controlling pH at a moderately acidic pH to a moderately basic pH include, but are not limited to, such compounds as phosphate, acetate, citrate, arginine, TRIS, and histidine. "TRIS" refers to 2-amino-2-hydroxymethyl-1,3,-propanediol, and to any

pharmacologically acceptable salt thereof. Preferable buffers are phosphate buffers with saline or an acceptable salt.

The term "phosphate buffer" refers to solutions containing phosphoric acid or salts thereof, adjusted to a desired pH. Generally phosphate buffers are prepared from phosphoric acid, or a salt of phosphoric acid, including but not limited to sodium and potassium salts. Several salts of phosphoric acid are known in the art, such as sodium and potassium monobasic, dibasic, and tribasic salts of the acid. Salts of phosphoric acid are also known to occur as hydrates of the occurring salt. Phosphate buffers may cover a range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of at or about 6.0 to at or about 8.0, most preferably at or about pH 7.0.

The term "vial" or "container" refers broadly to a reservoir suitable for retaining FSH in solid or liquid form in a contained sterile state. Examples of a vial as used he rein include ampoules, cartridges, blister packages, or other such reservoir suitable for delivery of the FSH to the patient via syringe, pump (including osmotic), catheter, transdermal patch, pulmonary or transmucosal spray. Vials suitable for packaging products for parenteral, pulmonary, transmucosal, or transdermal administration are well known and recognized in the art.

The term "stability" refers to the physical, chemical, and conformational stability of FSH and LH in the formulations of the present invention (including maintenance of biological potency). Instability of a protein formulation may be caused by chemical degradation or aggregation of the protein molecules to form higher order polymers, by dissociation of the heterodimers into monomers, deglycosylation, modification of glycosylation, oxidation (particularly of the α-subunit) or any other structural modification that reduces at least one biological activity of an FSH polypeptide included in the present invention.

A "stable" solution or formulation, is one wherein the degree of degradation, modification, aggregation, loss of biological activity and the like, of proteins therein is acceptably controlled, and does not increase unacceptably with time. Preferably the formulation retains at least at or about 80% of the labelled FSH activity and at least at or about 80% of the labelled LH activity over a period of 6 months at a temperature of at or about 2-8°C, more preferably at or about 2-8°C, more preferably at or about 4-5°C. FSH activity can be measured using the Steelman-Pohley ovarian weight gain

bioassay[6]. LH activity can be measured using the seminal vesicle weight gain bioassay[10].

The term "treating" refers to the administration, follow up, management and/or care of a patient for which FSH and/or LH administration is desirable for the purpose of follicle or testicular stimulation or any other physiological response regulated by FSH and/or LH. Treating can thus include, but is not limited to, the administration of FSH and/or LH for the induction or improvement of sperm quality, stimulation of testosterone release in the male, or follicular development or for ovulation induction in the female.

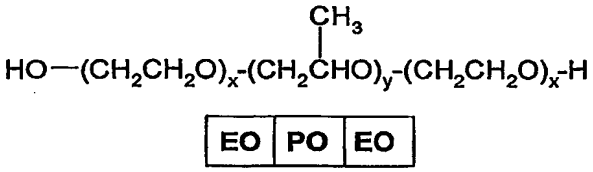
The expression "multi-dose use" is intended to include the use of a single vial, ampoule or cartridge of an FSH or LH formulation or a formulation of FSH and LH for more than one injection, for example 2, 3, 4, 5, 6 or more injections. The injections are preferably made over a period of at least at or about 12 hours, 24 hours, 48 hours, etc., preferably up to a period of at or about 12 days. The injections may be spaced in time, for example, by a period of 6, 12, 24, 48 or 72 hours.

A " salt" of a protein is an acid or base addition salt. Such salts are preferably formed between any one or more of the charged groups in the protein and any one or more physiologically acceptable, non-toxic cations or anions. Organic and inorganic salts include, for example, those prepared from acids such as hydrochloric, sulphuric, sulfonic, tartaric, fumaric, hydrobromic, glycolic, citric, maleic, phosphoric, succinic, acetic, nitric, benzoic, ascorbic, p-toluenesulfonic, benzenesulfonic, naphthalenesulfonic, propionic, carbonic, and the like, or for example, ammonium, sodium, potassium, calcium, or magnesium.

The inventors have found that Pluronic is a suitable excipient for preparing a stable formulation comprising LH or FSH as well as a mixture of LH and FSH. Moreover, the use of Pluronic allows the freeze dried formulation according to the present invention to be used both as a mono-dose as well as a multi-dose presentation, in particular where the reconstitution diluent contains a bacteriostatic selected from m-cresol or phenol.

The inventors have further found that when reconstituting the freeze dried formulation according to the invention with a diluent containing a bacteriostatic agent, such as *m*-cresol and phenol, no precipitation takes place. Precipitation, resulting in the formation of turbid or milky solutions occurs for instance when TWEEN 20 is used as a surfactant with m-cresol or phenol.

The Pluronic surfactants are block copolymers of ethylene oxide (EO) and propylene oxide (PO). The propylene oxide block (PO) is sandwiched between two ethylene oxide (EO) blocks.



Pluronic surfactants are synthesised in a two-step process:

1. A hydrophobe of the desired molecular weight is created by the controlled addition of propylene oxide to the two hydroxyl groups of propylene glycol; and

2. Ethylene oxide is added to sandwich the hydrophobe between hydrophilic groups.

In Pluronic® F77, the percentage of polyoxyethylene (hydrophile) is 70%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 2,306 Da.

In Pluronic F87, the percentage of polyoxyethylene (hydrophile) is 70%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 2,644 Da.

In Pluronic F88, the percentage of polyoxyethylene (hydrophile) is 80%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 2,644 Da.

In Pluronic F68, the percentage of polyoxyethylene (hydrophile) is 80%, and the molecular weight of the hydrophobe (polyoxypropylene) is approximately 1,967 Da.

Typical properties of Pluronic F77 are listed below:
Average Molecular Weight: 6600;
Melt/pour point: 48°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 480 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 47.0
0.01% Conc. : 49.3
0.001% Conc.: 52.8
Interfacial tension, dynes/cm @ 25°C vs. Nujol;
0.1% Conc. : 17.7
0.01% Conc. : 20.8
0.01% Conc. : 25.5
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 100
Ross Miles, 0.1%, mm @ 26°C: 47
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 25

Typical properties of Pluronic F87 are listed below:
Average Molecular Weight: 7700;
Melt/pour point: 49°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 700 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 44.0
0.01% Conc. : 47.0
0.001% Conc.: 50.2
Interfacial tension, dynes/cm @ 25°C vs Nujol;
0.1% Conc. : 17.4
0.01% Conc. : 20.3
0.01% Conc. : 23.3
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 80
Ross Miles, 0.1%, mm @ 26°C: 37
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 24

Typical properties of Pluronic F88 are listed below:
Average Molecular Weight: 11400;
Melt/pour point: 54°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 2300 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 48.5
0.01% Conc. : 52.6
0.001% Conc.: 55.7
Interfacial tension, dynes/cm @ 25°C vs Nujol;
0.1% Conc. : 20.5
0.01% Conc. : 23.3
0.01% Conc. : 27.0
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 80
Ross Miles, 0.1%, mm @ 26°C: 37
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 28

Typical properties of Pluronic F68 are listed below:
Average Molecular Weight: 8400;
Melt/pour point: 52°C ;
Physical Form @ 20°C : solid;
Viscosity (Brookfield) cps: 1000 [liquids at 25°C, pastes at 60°C and solids at 77°C];
Surface tension, dynes/cm @ 25°C;
0.1% Conc. : 50.3
0.01% Conc. : 51.2
0.001% Conc.: 53.6
Interfacial tension, dynes/cm @ 25°C vs Nujol;
0.1% Conc. : 19.8
0.01% Conc. : 24.0
0.01% Conc. : 26.0
Draves Wetting, Seconds 25°C
1.0% Conc.: > 360
0.1% Conc.: > 360
Foam Height
Ross Miles, 0.1%, mm @ 50°C: 35
Ross Miles, 0.1%, mm @ 26°C: 40
Dynamic, 0.1%, mm @ 400 ml/min: > 600
Cloud point in aqueous solution, °C
1% Conc.: >100
10% Conc.: >100
HLB (hydrophile-lipophile balance): 29

Such surfactants are commercially available from BASF Corporation Ludwigshafen, Germany.

Other polymers having properties similar to those listed above may also be used in the formulations of the invention. The preferred surfactant is Pluronic F68, and surfactants having similar properties.

The surfactant, e.g. Pluronic F 68 (also available as Poloxamer 188), is preferably present in the freeze dried formulation at a concentration of at or about 0.001 to at or about 0.1 mg per mg of the total formulation, more preferably at or about 0.01 to at or about 0.075 mg/mg.

Preferably the concentration of Pluronic, particularly Pluronic F68, in the reconstituted formulations is at or about 0.01 mg/ml to at or about 1 mg/ml, more preferably at or about 0.05 mg/ml to at or about 0.5 mg/ml, more particularly preferably at or about 0.2 mg/ml to at or about 0.4 mg/ml, most preferably at or about 0.1 mg/ml.

The follicle-stimulating hormone (FSH) within the freeze-dried formulation is preferably present at a concentration (w/w) of at or about 0 .1 to 10 µg/mg of the total formulation. In one embodiment, the follicle-stimulating hormone (FSH) is present at a concentration of at or about 0.3 to 5 µg/mg of the total formulation. In a further embodiment the follicle-stimulating hormone (FSH) is present at a concentration of at or about 0.37 to 2 µg/mg of the total formulation.

The luteinising hormone (LH) within the freeze-dried formulation is preferably present at a concentration of at or about 0.1 to 3 µg/mg of the total formulation. In one embodiment, the luteinising hormone (LH) is present at a concentration of at or about 0.1 to 1 µg/mg of the total formulation. In a further embodiment, the luteinising hormone (LH) is present at a concentration of at or about 0.1 to 0.6 µg/mg of the total formulation.

In the reconstituted formulations comprising FSH, preferably the concentration of FSH in the formulation is at or about 150 IU/ml to at or about 2,000 IU/ml, more preferably at or about 300 IU/ml to at or about 1,500 IU/ml, more particularly preferably at or about 450 to at or about 750, most preferably at or about 600 IU/ml.

In the reconstituted formulations comprising LH, preferably the LH concentration in the formulation is at or about 50 IU/ml to at or about 2,000 IU/ml, more preferably at or about 150 to at or about 1,500 IU/ml, more particularly preferably at or about 300 IU/ml to at or about 750 IU/ ml, particularly preferably 625 IU/ml.

In formulations comprising both FSH and LH, the ratio of FSH to LH (FSH:LH, IU:IU, FSH measured with rat ovarian weight gain assay and LH measured with rat seminal vesicle weight gain assay) is preferably within the range of at or about 6:1 to at or about 1:6, more preferably at or about 4:1 to at or about 1:2, more particularly preferably at or about 3:1 to at or about 1:1. Particularly preferred ratios are 1:1 and 2:1.

Preferably the FSH and LH are produced recombinantly, particularly preferably they are produced in Chinese hamster ovary cells transfected with a vector or vectors comprising DNA coding for the human glycoprotein alpha-subunit and the beta-subunit of FSH or LH. DNA encoding the alpha and beta-subunits may be present on the same or different vectors.

Recombinant FSH and LH have several advantages over their urinary counterparts. Culture and isolation techniques using recombinant cells permit consistency between batches. In contrast, urinary FSH and LH vary greatly from batch to batch in such characteristics as purity, glycosylation pattern, sialylation and oxidation of the subunits. Due to greater batch-to-batch consistency and purity of recombinant FSH and LH, the hormones can be readily identified and quantified using techniques such as isoelectric focussing (IEF). The ease with which recombinant FSH and LH can be identified and quantified permits the filling of vials by mass of hormone (fill-by-mass) rather than filling by bioassay.

Preferably the freeze dried formulations of the present invention have a buffer, preferably a phosphate buffer, with preferred counterions being sodium or potas sium ions. Phosphate saline buffers are well known in the art, such as Dulbecco's Phosphate buffered saline. Buffer concentrations in total solution can vary between at or about 5mM, 9.5mM, 10mM, 50mM, 100mM, 150mM, 200mM, 250mM, and 500mM. Preferably the buffer concentration is at or about 10mM. Particularly preferred is a buffer 10 mM in phosphate ions with a pH of 7.0.

Preferably the buffer is adjusted in such a way that the reconstituted formulations of the freeze dried formulations of the present invention have a pH between at or about 6.0 and at or about 8.0, more preferably at or about 6.8 to at or about 7.8, including about pH 7.0, pH 7.2, and 7.4.

Preferably the buffer is adjusted in such a way that the reconstituted formulations of mixtures of FSH and LH of the present invention have pH between at or about 6.0 and at or about 9.0, more preferably at or about 6.8 to at or about 8.5, including about pH 7.0, pH 8.0, and 8.2, most preferably at or about pH 8.0.

In order to provide for an injectable, the freeze dried formulations of the present invention are reconstituted using a suitable solvent. A preferred solvent is water for injection. Liquid formulations may be single dose or multi-dose. Those reconstituted liquid FSH, LH or FSH/LH formulations of the invention that are intended for multi-dose use preferably comprise a bacteriostatic within the solvent for reconstitution. Suitable bacteriostatic agents include phenol, *m*-cresol, *p*-cresol, *o*-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), thymol, benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal. Particularly preferred are phenol, benzyl alcohol and *m*-cresol, more preferred are phenol and *m*-cresol, most preferred is *m*-cresol. The bacteriostatic agent is used in an amount that will yield a concentration that is effective to maintain the reconstituted formulation essentially bacteria free (suitable for injection) over the multi-dose injection period, which may be at or about 12 or 24 hours to at or about 12 or 14 days, preferably at or about 6 to at or about 12 days. The bacteriostatic is preferably present in a concentration of at or about 0.1% (mass bacteriostatic/mass of solvent) to at or about 2.0%, more preferably at or about 0.2% to at or about 1.0%. In the case of benzyl alcohol, particularly preferred is a concentration of 0.9%). In the case of phenol, particularly preferred is at or about 0.5%. In the case of *m*-cresol, particularly preferred is a concentration of at or about 0.3 % (e.g. at or about 3 mg/ml in WFI).

In a specific embodiment, the invention provides a freeze dried formulation for reconstitution, preferably for multi-dose use, comprising FSH or a variant thereof and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, preferably Pluronic F 68.

In a further specific embodiment, the invention provides a freeze dried formulation for reconstitution, preferably for multi-dose use, comprising LH, a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, preferably Pluronic F68.

In a further specific embodiment, the invention provides a freeze dried formulation, preferably for multi-dose use, comprising FSH and LH, a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, preferably Pluronic F68. Preferably the FSH and LH are present in a ratio (FSH:LH) of at or about 2:1 to at or about 1:1.

In a further specific embodiment, the invention provides a method for manufacturing a freeze dried formulation, preferably for multi-dose use after reconstitution, comprising forming a mixture of FSH or a variant thereof with a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and subjecting said mixture to lyophilisation.

In a further specific embodiment, the invention provides a method for manufacturing a freeze dried formulation, preferably for multi-dose use after reconstitution, comprising forming a mixture of LH with a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and subjecting said mixture to lyophilisation.

In a further specific embodiment, the invention provides a method for manufacturing a freeze dried formulation, preferably for multi-dose use after reconstitution, comprising forming a mixture of FSH and LH as well as a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and subjecting said mixture to lyophilisation.

In yet another preferred embodiment, the invention provides a method for manufacturing a packaged pharmaceutical composition comprising dispensing a freeze dried mixture comprising FSH and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

In yet another preferred embodiment, the invention provides a method for manufacturing a packaged pharmaceutical composition comprising dispensing a

freeze dried mixture comprising LH and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 into a container.

In yet another preferred embodiment, the invention provides a method for manufacturing a packaged pharmaceutical composition comprising dispensing a freeze dried mixture comprising FSH as well as LH and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68 into a container.

In yet another preferred embodiment, the invention provides an article of manufacture for human pharmaceutical use, comprising a first container or vial comprising freeze dried FSH or an FSH variant and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68. A second container or vial contains a diluent for reconstitution, preferably water and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol.

In yet another preferred embodiment, the invention provides an article of manufacture for human pharmaceutical use, comprising a first container or vial comprising freeze dried LH or an LH variant and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68. A second container or vial contains a diluent for reconstitution, preferably water and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol.

In yet another preferred embodiment, the invention provides an article of manufacture for human pharmaceutical use, comprising a first container or vial comprising freeze dried FSH or an FSH variant as well as LH or an LH variant and a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68. A second container or vial contains a diluent for reconstitution, preferably water and a bacteriostatic selected from m-cresol and phenol, preferably m-cresol.

In a particularly preferred embodiment, the solvent for reconstitution comprises *m*-cresol. The inventors have found that freeze dried formulations comprising Pluronic F68 do not precipitate when reconstituted with a diluent containing *m*-cresol, a problem observed with other surfactants, e.g. Tween.

The freeze dried formulations of the invention may be kept for at least at or about 6 months, 12 months or 24 months. Under preferred storage conditions, before the first use, the formulations are kept away from bright light (preferably in the dark), at

temperatures of at or about 25, preferably of at or about 2-8°C, more preferably at or about 4-5°C.

After the first use of a reconstituted multi-dose formulation it may be kept and used for at least at or about 24 hours, preferably at least at or about 4, 5 or 6 days, more preferably for up to 12 or 14 days. After the first use the formulation is preferably stored at below room temperature (i.e. below at or about 25 °C), more preferably below at or about 10°C, more preferably at or about 2-8°C, most preferably at or about 5-0°C.

Preferably the freeze dried formulations of the invention contain an antioxidant, such as methionine, sodium bisulfite, salts of ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), and butylated hydroxy anisole (BHA). Most preferred is methionine. The antioxidant prevents oxidation of FSH and LH (particularly the α-subunit).

The antioxidant, e.g. methionine is preferably present at a concentration of at or about 0.001 to at or about 0.1 mg per mg of total formulation, more preferably at or about 0.01 to at or about 0.075 mg/mg.

In the reconstituted formulation, methionine is preferably present at a concentration of at or about 0.01 to at or about 1.0 mg/ml, more preferably at or about 0.05 to at or about 0.5 mg/ml, most preferably at or about 0.1 mg/ml.

Preferably the freeze dried formulations formulations of the invention contain a mono- or disaccharide or a sugar alcohol as stabiliser and tonicity adjusting agent, such as sucrose, dextrose, lactose, mannitol and/or glycerol. Most preferred is sucrose. In the reconstituted formulation, sucrose is present at or about 60 mg/ml.

As noted above, the invention provides freeze dried formulations for single use and multi-dose use. The formulations of the invention are suitable for pharmaceutical or veterinary use.

As noted above, in a preferred embodiment, the invention provides an article of manufacture, comprising packaging material and a vial comprising freeze dried FSH or an FSH variant, LH or an LH variant, or FSH and LH, Pluronic F68. The bacteriostatic within the second container including the diluent is selected from

phenol and *m*-cresol, optionally with further excipients, wherein said packaging material comprises written material which indicates that such solution may be held over a period of twenty-four hours or greater after the first use.

The range of protein hormone in the formulations of the invention includes amounts yielding upon reconstitution, concentrations from about 1.0 µg/ml to about 50 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods. The protein hormone concentration is preferably at or about 5.0 µg/ml to at or about 2 mg/ml, more preferably at or about 10 µg/ml to at or about 1 mg/ml, most preferably at or about 50 µg/ml to at or about 200 µg/ml.

Preferably the formulations of the invention retain at least at or about 80% of the FSH activity and/or LH activity at the time of packaging over a period of 24 months (before the first use). FSH activity can be measured using the Steelman-Pohley ovarian weight gain bioassay[5]. LH activity can be measured using the rat seminal vesicle weight gain bioassay.

The formulations of the present invention can be prepared by a process which comprises mixing FSH or an FSH variant, LH or an FSH variant, or a mixture of FSH and LH and Pluronic F68 as well as further excipients like an antioxidant and/or a buffer and subjecting the mixture to a lyophilisation. Mixing the components and lyophilising them is carried out using conventional procedures. To prepare a suitable formulation, for example, a measured amount of FSH or FSH variant, LH or LH variant or a mixture of FSH and LH is combined with Pluronic F68 and the resulting mixture is lyophilized and then dispensed into vials, ampoules or cartridges. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimised for the concentration and means of administration used.

The reconstituted formulations obtained from the freeze dried formulations of the invention can be administered using recognized devices. Examples comprising these single vial systems include pen-injector devices for delivery of a solution such as EasyJect®, Gonal-F® Pen, Humaject®, NovoPen®, B-D®Pen, AutoPen®, and OptiPen®.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product may be used. The packaging material of the present invention provides instructions to the patient to reconstitute the freeze dried formulation of the invention in the aqueous diluent to form a solution and to use the solution over a period of twenty-four hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution may be stored after first use for a period of twenty-four hours or greater, preferably for up to 12 or 14 days. The presently claimed products are useful for human pharmaceutical product use.

The following examples are provided merely to further illustrate the preparation of the formulations and compositions of the invention. The scope of the invention shall not be construed as merely consisting of the following examples.

Examples

The recombinant gonadotropins (FSH / LH) of the present examples have been prepared by expression in CHO (Chinese Hamster Ovary) cells, transformed with the corresponding recombinant DNA, according to the techniq ue described in European patents EP 160699 and EP 211894.

The other substances used in the examples are the following:

- Sucrose extra pure Merck code 1.07653
- Sodium dihydrogen phosphate monohydrate (following indicated as NaH_2PO_4 H_2O) Merck code 1.06346
- Di-Sodium hydrogen phosphate dihydrate (following indicated as Na_2HPO_4 $2H_2O$) Merck code 1.06580
- Pluronic F-68 BASF Corporation Ludwigshafen code 8176061
- L-Methionine Rexim
- Water for injection
- Ortho Phosphoric Acid 85% extra pure Merck code 1.00563
- Ortho Phosphoric acid (17% w/w approx.) solution
- Sodium Hydroxide pellets extra pure Merck code 1.06498

FSH and LH freeze dried multidose formulation

Two freeze dried formulations A and B having the following compositions have been prepared:

Formulation A

FSH	µg 32.75 (450 I.U.)
LH	µg 9.0 (225 I.U.)
Sucrose	mg 15.0
NaH_2PO_4 H_2O	mg 0.052
Na_2HPO_4 $2H_2O$	mg 0.825
Pluronic F-68	mg 0.05
L-Methionine	mg 0.05

Formulation B

FSH	µg 65.5 (900 I.U.)
LH	µg 18.0 (450 I.U.)
Sucrose	mg 30.0
NaH_2PO_4 H_2O	mg 0.104
Na_2HPO_4 $2H_2O$	mg 1.65
Pluronic F68	mg 0.10

L-Methionine mg 0.10

The manufacturing process consists in mixing the drug substance directly with the ingredients, filtrating the solution obtained and lyophilising the filtrated.

A description of each step of the process is given in the following :

- add in a tared container WFI, di-sodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate monohydrate, Sucrose, Pluronic F68 at 5% and L-methionine and stir for 10 minutes until complete dissolution.
- check the pH and eventually correct it to pH 7.00 ± 0.2 with NaOH 10% or diluted H_3PO_4
- add FSH and LH to the above prepared mixture and gently stir the solution obtained for 10 minutes.
- check the pH again and eventually adjust it to 7.0 ± 0.1 with 10% NaOH or diluted H_3PO_4.
- filter the solution with a 0.22 µm Durapore membrane with a filtration ratio not less than 15g/cm2, under Nitrogen gas flow with a pressure not higher than 1.5 atm.
- collect the solution in a previously sterilised flask.
- fill the filtered solution into the glass container, seat the stopper and place the filled vials into a stainless steel tray.
- load the trays into the freeze dryer and lyophilise the product using the following freeze drying cycle :
  - equilibrate at +4°C for about 20 mins.
  - bring the shelves temperature at -25°C and maintain for 2 hours.
  - bring the shelves temperature at -15°C and maintain for 1 hour.
  - bring the shelves temperature at -45°C and maintain for 3 hours.
  - bring condenser temperature at -65°C.
  - apply vacuum to the chamber.
  - When the vacuum reaches a value of $7x10^{-2}$ mBar raise shelf temperature up to -10°C and maintain for 14 hours.
  - raise the shelf temperature up to +35°C in 8 hours and maintain up to the end of the cycle (14 hours).
  - break the vacuum allowing dry nitrogen into the chamber.
  - perform the stoppering by automatic system of the freeze dryer.
  - seal the stoppered vials with the appropriate flip-off caps.

The formulations A and B have been stored at 25 ± 2°C, and tested for stability and biological activity as pointed out below. Prior to analysing the compositions, they are reconstituted using water for injection comprising 0.3% of m-Cresol as bacteriostatic agent.

The stability and biological activity values were determined as follows :

- In vivo assay for FSH : The formulation was tested for FSH activity using the Steelman-Pohley ovarian weight gain bioassay
- In vivo assay for LH : The formulation was tested for LH activity using the rat seminal vesicle weight gain bioassay.
- Assay of oxidised alpha-subunit : The percentage of oxidised alpha-subunit was measured by a reverse phase HPLC (RP-HPLC) method.
- Evaluation of free subunit (rFSH + rLH) : The percentage of free subunit was evaluated by SDS-PAGE.
- Evaluation of aggregates : The percentage of aggregates was evaluated by SDS-PAGE as described above for evaluation of free subunit.

The biological tests have been performed in compliance with the regulations of the European Pharmacopeia. In particular the tests are reported in the "Menotropin" monography.

Table 1 summarizes the results of the analytical tests related to stability and biological activity of formulation A. The values were determined at 4 check-points : at time zero, after 1 month, 3 months and 6 months of storage, at a storage temperature of 25 ± 2°C.

TABLE 1

TEST	TIME ZERO	1 MONTHS	3 MONTHS	6 MONTHS
Biological activity I.U. FSH	416	420	415	417
Biological activity I.U. LH	276	250	259	270
% oxidised product	1.95	1.81	1.95	1.57
% dimers/aggregates	<2	<2	<2	<2
% free subunits	<5	<5	<5	<5

Table 2 summarizes the results of the analytical tests related to stability and biological activity of formulation B. The values were determined at 4 check-points : at time zero, after 3 month, 6 months and 9 months of storage, at a storage temperature of 25 ± 2°C.

TABLE 2

TEST	TIME ZERO	3 MONTHS	6 MONTHS	9 MONTHS
Biological activity I.U. FSH	821	850	830	838
Biological activity I.U. LH	570	564	580	622
% oxidised product	1.0	0.9	1.0	1.0
% dimers/aggregates	<2	<2	<2	<2
% free subunits	<5	<5	<5	<5

From TABLE 1 and 2 it may be concluded that the biological activity of formulations A and B is well conserved after 9 months of storage. The formulations have a high stability.

The high stability is not affected by large amounts of recombinant FSH and recombinant LH.

Sequences :

SEQ ID NO. 1: human glycoprotein α-subunit;

SEQ ID NO. 2: hFSH β-subunit

SEQ ID NO. 3: hFSH β-subunit variant 1

SEQ ID NO. 4: hFSH β-subunit variant 2

SEQ ID NO. 5: hFSH β-subunit variant 3

SEQ ID NO. 6: hLH β-subunit

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Claims

1. A freeze-dried formulation comprising follicle-stimulating hormone (FSH) or a variant thereof, as well as a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

2. A freeze-dried formulation comprising luteinising hormone (LH) or a variant thereof, as well as a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

3. A freeze-dried formulation comprising follicle-stimulating hormone (FSH) or a variant thereof as well as luteinising hormone (LH) or a variant thereof, as well as a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68.

4. The freeze dried formulation according to any of claims 1 to 3, wherein the surfactant is Pluronic F68.

5. The freeze dried formulation according to any of claims 1 to 4, wherein the follicle-stimulating hormone is human follicle-stimulating hormone and/or the luteinising hormone (LH) is human luteinising hormone (LH).

6. The freeze dried formulation according to claim 5, wherein the follicle-stimulating hormone is urinary human follicle-stimulating hormone and/or the luteinising hormone (LH) is urinary human luteinising hormone (LH).

7. The freeze dried formulation according to any of claims 1 to 4, wherein the follicle-stimulating hormone is recombinant human follicle-stimulating hormone and/or the luteinising hormone (LH) is recombinant human luteinising hormone (LH).

8. The freeze dried formulation according to any of the preceding claims, wherein the follicle-stimulating hormone (FSH) is present at a concentration (w/w) of at or about 0.1 to 10 µg/mg of the total formulation.

9. The freeze dried formulation according to claim 8, wherein the follicle-stimulating hormone (FSH) is present at a concentration of at or about 0.3 to 5 µg/mg of the total formulation.

10. The freeze dried formulation according to claim 9, wherein the follicle-stimulating hormone (FSH) is present at a concentration of at or about 0.37 to 2 µg/mg of the total formulation.

11. The freeze dried formulation according to any of the preceding claims, wherein the luteinising hormone (LH) is present at a concentration of at or about 0.1 to 3 µg/mg of the total formulation.

12. The freeze dried formulation according to claim 11, wherein the luteinising hormone (LH) is present at a concentration of at or about 0.1 to 1 µg/mg of the total formulation.

13. The freeze dried formulation according to claim 12, wherein the luteinising hormone (LH) is present at a concentration of at or about 0.1 to 0.6 µg/mg of the total formulation.

14. The freeze dried formulation according to any of the preceding claims, wherein the ratio of FSH to LH is within the range of at or about 6:1 to at or about 1:6.

15. The freeze dried formulation according to claim 14, wherein the ratio of FSH to LH is within the range of at or about 4:1 to at or about 1:2.

16. The freeze dried formulation according to claim 15, wherein the ratio of FSH to LH is within the range of at or about 3:1 to at or about 1:1.

17. The freeze dried formulation according to claim 16, wherein the ratio of FSH to LH is within the range of at or about 2:1 and 1:1.

18. The freeze dried formulation according to any of the preceding claims, further comprising sucrose.

19. The freeze dried formulation according to any of the preceding claims , further comprising methionine.

20. The freeze dried formulation according to any of the preceding claims, further comprising a phosphate buffer.

21. The freeze dried formulation according to any of the preceding claims, comprising the following ingredients: rFSH, rLH, Pluronic F68, sucrose, methionine, a phosphate buffer.

22. The freeze dried formulation according to any of the preceding claims, comprising 32.75 µg of recombinant FSH, 9.0 µg of recombinant LH, 15.0 mg of sucrose, 0.052 mg of NaH_2PO_4 H_2O, 0.825 mg of Na_2HPO_4 $2H_2O$, 0.05 mg of Pluronic F68 and 0.05 mg of L-methionine.

23. The freeze dried formulation according to any of claims 1 to 21, comprising 65.5 µg of recombinant FSH, 18.0 µg of recombinant LH, 30.0 mg of sucrose, 0.104 mg of NaH_2PO_4 H_2O, 1.65 mg of Na_2HPO_4 $2H_2O$, 0.10 mg of Pluronic F68 and 0.10 mg of L-methionine.

24. An article of manufacture comprising a first container filled with a freeze dried formulation according to any claims from 1 to 23 and a second container comprising a solvent for reconstitution.

25. An article of manufacture according to claim 24, whereby the second container comprises an aqueous diluent containing m-cresol.

26. A method for manufacturing a freeze dried formulation according to any of claims 1 to 23, comprising the step of forming a mixture of FSH with or without LH, or LH alone LH as well as a surfactant selected from Pluronic® F77, Pluronic F87, Pluronic F88 and Pluronic F68, and subjecting the mixture to a lyophilisation.

27. A method according to claim 25, wherein the surfactant is Pluronic F68.

Field of Invention

The invention relates to the field of pharmaceutical formulations of follicle-stimulating hormone (FSH), luteinising hormone (LH) and mixtures of FSH and luteinising hormone (LH), and to methods of producing such formulations.

SEQUENCE LISTING

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<213> Homo sapiens

<400> 1

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Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro
1               5                  10                  15

Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys
            20                  25                  30

Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu
        35                  40                  45

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser
    50                  55                  60

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Val Glu Asn His Thr Ala
65                  70                  75                  80

Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser
                85                  90
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<210> 2

<211> 129

<212> PRT

<213> Homo sapiens

<400> 2

Met Lys Thr Leu Gln Phe Phe Phe Leu Phe Cys Cys Trp Lys Ala Ile
1 5 10 15

Cys Cys Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys
20 25 30

Glu Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr Trp Cys Ala Gly
35 40 45

Tyr Cys Tyr Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg Pro Lys
50 55 60

Ile Gln Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu Thr Val Arg
65 70 75 80

Val Pro Gly Cys Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro Val
85 90 95

Ala Thr Gln Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr Asp Cys
100 105 110

Thr Val Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
115 120 125

Glu

<210> 3

<211> 108

<212> PRT

<213> Homo sapiens

<400> 3

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys Glu Glu

1 5 10 15

Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys
20 25 30

Tyr Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln
35 40 45

Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu Thr Val Arg Val Pro
50 55 60

Gly Cys Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro Val Ala Thr
65 70 75 80

Gln Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr Asp Cys Thr Val
85 90 95

Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe Gly Glu
100 105

<210> 4

<211> 106

<212> PRT

<213> Homo sapiens

<400> 4

Asn Ser Cys Glu Leu Thr Asn Ile Ala Ile Glu Lys Glu Glu Cys Arg
1 5 10 15

Phe Cys Ile Ser Ile Asn Thr Trp Cys Ala Gly Tyr Cys Tyr Thr Arg
20 25 30

Asp Leu Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln Lys Thr Cys
35 40 45

Thr Phe Lys Glu Leu Val Tyr Glu Thr Val Arg Val Pro Gly Cys Ala
50 55 60

His His Ala Asp Ser Leu Tyr Thr Val Pro Val Ala Thr Gln Cys His
65 70 75 80

Cys Gly Lys Cys Asp Ser Asp Ser Thr Asp Cys Thr Val Arg Gly Leu
85 90 95

Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met
100 105

<210> 5

<211> 110

<212> PRT

<213> Homo sapiens

<400> 5

Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala Ile Glu Lys Glu Glu
1 5 10 15

Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr Trp Cys Ala Gly Tyr Cys
20 25 30

Tyr Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg Pro Lys Ile Gln
35 40 45

Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu Thr Val Arg Val Pro
50 55 60

Gly Cys Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro Val Ala Thr
65 70 75 80

Gln Cys His Cys Gly Lys Cys Asp Ser Asp Ser Thr Asp Cys Thr Val
85 90 95

Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe Gly Glu Met Lys
100 105 110

<210> 6

<211> 112

<212> PRT

<213> Homo sapiens

<400> 6

Ser Arg Glu Pro Leu Arg Pro Trp Cys His Pro Ile Asn Ala Ile Leu
1 5 10 15

```
Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr
            20                  25                  30

Ile Cys Ala Gly Tyr Cys Pro Thr Met Arg Val Leu Gln Ala Val Leu
        35                  40                  45

Pro Pro Leu Pro Gln Val Cys Thr Tyr Arg Asp Val Arg Phe Glu Ser
    50                  55                  60

Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asp Pro Val Val Ser Phe
65                  70                  75                  80

Pro Val Ala Leu Ser Cys Arg Cys Gly Pro Cys Arg Arg Ser Thr Ser
                85                  90                  95

Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp His Pro Gln
            100                 105                 110
```